

LM cells, manipulating the membrane phospholipid composition modulated adenylate cyclase activity independently of changes in fluorescence polarization, a measure of membrane fluidity (25). A change in receptor synthesis or degradation by testosterone hemisuccinate is unlikely to account for its effects, because these effects occurred rapidly and included a shift in receptor affinity.

Our observations indicate possible pitfalls in investigating the effects of proposed receptor modulators on the actions of angiotensin or other ligands. For example, approximately half of the 70 steroids we added to adrenal cells inhibited angiotensin binding (26). However, in all of those experiments the steroids remained in the medium during the binding assay. Tested in the same way, testosterone hemisuccinate also inhibited angiotensin binding. However, in cells washed thoroughly after incubation with this steroid, angiotensin binding and stimulation of aldosteronogenesis were markedly augmented. Putative modulators may exert complex positive and negative effects or may require a latent period to influence receptors. Investigators testing substances for modification of membrane-related processes should take these possibilities into account.

Our ability to enhance the activity of angiotensin receptors by naturally occurring steroids in vitro offers a new way to test for putative mediators of low renin essential hypertension. This condition is usually marked by normal aldosterone secretion in the face of subnormal levels of renin (27) and may be the result of sensitization of adrenal glomerulosa by circulating substances. We suggest that angiotensin potentiators in body fluids from patients with low renin essential hypertension can be sought by a brief, transient exposure of adrenal cells to the fluids, as well as by adding the fluids to the final assay mixture.

JOHN E. CARROLL

THEODORE L. GOODFRIEND

William S. Middleton Memorial  
Veterans Administration Hospital and  
Departments of Medicine and  
Pharmacology, University of  
Wisconsin, Madison 53705

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## Perinatal Dopamine-Related Drugs Demasculinize Rats

**Abstract.** Administration of haloperidol, a common neuroleptic, to pregnant or lactating rats impaired the masculine sex behavior of their male offspring. Prenatal haloperidol did not affect testosterone concentrations in fetuses. Maternal administration of apomorphine, a dopamine agonist, and of  $\alpha$ -methyl-p-tyrosine, an inhibitor of dopamine synthesis, also demasculinized male offspring. In both experiments other behaviors and developmental milestones were unaffected. Perinatal haloperidol, apomorphine, and  $\alpha$ -methyl-p-tyrosine did not lower testosterone in adulthood. These drugs may act directly on neurons that control masculine behavior without lowering testosterone prenatally or in adulthood.

Perinatal administration of certain drugs or hormones produces lasting impairment of reproductive function and sexual behavior in male rodents (1-4). Several of these agents appear to exert their demasculinizing effects by reducing the concentration of testosterone perinatally or in adulthood (2, 3, 5, 6). While high concentrations of testosterone during the week before and the week after birth appear to be critical for sexual differentiation (7), the mechanisms by which testosterone exerts its masculinizing effects have not been identified.

One possible mechanism of testosterone action involves a direct effect on developing neurons through alterations in growth, synaptogenesis, receptors, or enzyme activity. Monoaminergic neurons have been shown to regulate the expression of sex behavior in adulthood, with dopamine (DA) facilitating (8, 9) and serotonin inhibiting masculine behavior (9, 10). Alterations in monoaminergic activity may also play a role in the developmental demasculinizing effects noted above. Because DA is the only monoamine shown to facilitate masculine behavior in adulthood, we investigated the effects of several perinatally administered drugs that affect DA transmission.

Haloperidol (HAL) blocks DA recep-

tors preferentially though not exclusively and crosses placental and lactational barriers (11). Rosengarten and Friedhoff (12) reported that administration of HAL to pregnant rats depressed binding of [<sup>3</sup>H]spiroperidol in the brains of their offspring as late as 60 days of age. Behavioral responsiveness of the offspring to apomorphine (APO), a DA agonist, was also depressed by prenatally administered HAL. On the other hand, neonatal treatment with HAL (through lactation) had the opposite effect, increasing [<sup>3</sup>H]spiroperidol binding and behavioral responsiveness to APO. This is similar to the supersensitivity to DA seen in adult animals after long-term treatment with neuroleptics. Because DA facilitates adult masculine sex behavior, we hypothesized that prenatally administered HAL would impair masculine behavior in adulthood, that neonatal treatment with HAL would facilitate it, and that combined pre- and neonatal treatments would tend to cancel these effects.

Ten Long-Evans female rats were mated and divided into two groups that were injected intraperitoneally with HAL (2.5 mg/kg) or saline from day 7 of gestation until day 21 postpartum, except on the day of birth. Treatment parameters were the same as those shown by Rosengarten and Friedhoff (12) to

affect receptor binding and behavioral stereotypy. Half the pups of each litter were cross-fostered on the day of birth to a mother receiving the opposite treatment. Thus four treatment groups were formed: one receiving HAL both pre- and postnatally (HH; 8 males and 7 females), one receiving HAL prenatally only (HS; 7 males and 6 females), one receiving HAL postnatally only (SH; 9 males and 12 females), and one receiving saline only (SS; 10 males and 11 females).

We tested male offspring for masculine sexual behavior with a receptive female at 65, 72, and 79 days of age (13). In test 1 (day 65), males in all three drug groups (HH, HS, and SH) had significantly fewer ejaculations than the control animals (Fig. 1a). However, in test 3 (day 79), only prenatally treated animals (groups HH and HS) exhibited a significant deficit. There was no statistically significant difference among groups in the percentage of animals achieving at least one intromission. Thus the "arousal mechanism" leading to the onset of copulation (14) was not impaired. However, the perinatal drug treatments significantly reduced the probability of ejaculation among males that intromitted (50, 53, 71, and 87 percent in groups HH, HS, SH, and SS, respectively) [ $\chi^2(3) = 14.01, P < 0.01$ ] (15). This suggests a deficit in

the "copulatory mechanism," which is thought to summate the effects of repeated intromissions until an ejaculation is triggered (14). Among animals that had achieved at least one ejaculation there were no significant treatment-related differences in any copulatory measure. Thus perinatal HAL reduced the number of males able to achieve ejaculation once they began intromitting.

There were no statistically significant differences in feminine sexual behavior among groups of female offspring (16). Also unaffected were open field ambulation (in both sexes) at 20, 40, and 80 days of age; age at eye opening or testicular descent; and HAL-induced catalepsy at 90 days of age (17). The only statistically significant difference in body weight was observed on postnatal day 8, when HH and HS females were lighter than SS and SH females [ $F(3, 27) = 4.31, P < 0.05$ ]. Thus, the drug treatment did not debilitate the pups or delay their maturation. Furthermore, since HH and HS males exhibited similar deficits, drug-induced alteration of maternal behavior was not a major factor producing the deficits.

To determine whether a depression of fetal testosterone might have mediated the demasculinization, we treated additional groups of five females each with HAL (2.5 mg/kg) or saline on days 7 to

18 of gestation. Concentrations of testosterone (18) on day 18 [the day of peak testosterone (7)] were not significantly different between groups.

Since blocking DA receptors pre- or neonatally impaired male sex behavior in adulthood, we designed a second experiment to test the effects of facilitating DA transmission perinatally with APO. Furthermore, since HAL, in addition to blocking DA receptors, also increases DA synthesis, we wished to inhibit DA synthesis in another group of animals. Therefore, we administered  $\alpha$ -methyl-*p*-tyrosine methyl ester ( $\alpha$ -MT; 60 mg/kg to inhibit DA synthesis) to one group of mother rats, APO (1 mg/kg to facilitate DA transmission) to another, both  $\alpha$ -MT and APO to a third, and the saline vehicle to a fourth. Drugs were administered from day 13 of gestation to postnatal day 21, except on the day of birth; each litter received the same treatment pre- and postnatally. There were ten male offspring in the  $\alpha$ -MT group, ten in the APO group, nine in the group that received both  $\alpha$ -MT and APO, and nine in the control group.

To assess the relative importance of maturational age and sexual experience in any improvement in sexual behavior that might occur across the three tests, we tested half the males of each litter at 60, 75, and 90 days of age and half on days 90, 105, and 120. There were no statistically significant differences between the 60-day series and the 90-day series for any group; therefore, all animals in each treatment group were combined for further statistical analyses. As seen in Fig. 1b, control males achieved more ejaculations on tests 2 and 3 than did males in the three drug groups. Among males that ejaculated in test 3, control males ejaculated sooner than males in any of the drug groups; the drug groups did not differ significantly among themselves (Fig. 1c). As in the first experiment, the decreased number of ejaculations was related to a decreased probability of ejaculation in animals that intromitted (67, 55, 67, and 93 percent in the  $\alpha$ -MT, APO,  $\alpha$ -MT + APO, and saline groups, respectively) [ $\chi^2(3) = 10.94, P < 0.02$ ] rather than to a reduced probability of intromitting at all (19).

After being tested for sexual behavior, the animals were tested for APO-induced stereotypy and HAL-induced catalepsy (20). Scores on these measures did not differ significantly among groups and were not correlated with number of ejaculations. Furthermore, differences in sexual behavior could not be attributed to general malnutrition or to delayed development of the drug-treated ani-

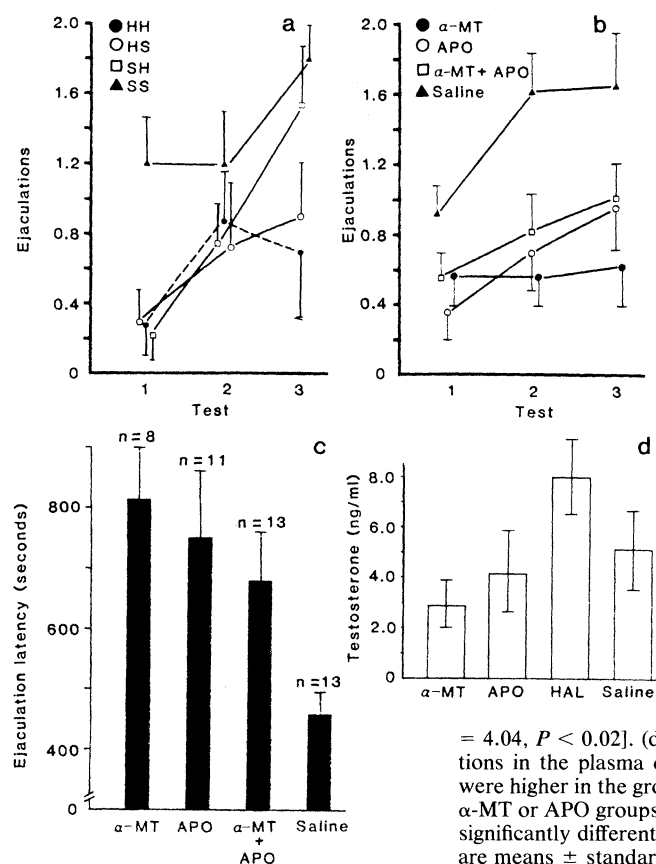


Fig. 1. Effects of perinatal drugs on sex behavior and testosterone concentrations in male rats. (a) Number of ejaculations per 30-minute test (experiment 1). In test 1 control animals had more ejaculations than any drug group [ $F(3, 30) = 5.12, P < 0.01$ ]. In test 3 the controls had more ejaculations than groups HH and HS [ $F(3, 30) = 3.31, P < 0.05$ ]. (b) Number of ejaculations per 30-minute test (experiment 2). In tests 2 and 3 control animals had more ejaculations than any drug group [ $F(3, 34) = 4.81, P < 0.01$  and  $F(3, 34) = 4.90, P < 0.01$ ]. (c) Ejaculation latencies. Controls had shorter latencies than any drug group ( $F(3, 30) = 4.04, P < 0.02$ ). (d) Testosterone concentrations in the plasma of 60-day-old rats. Levels were higher in the group given HAL than in the  $\alpha$ -MT or APO groups ( $P < 0.05$ ); no group was significantly different from the controls. Values are means  $\pm$  standard errors.

mals. As a measure of motor development, pups were tested on days 3 and 7 for latency to right themselves when placed on their backs; there were no significant differences among groups. Nor were there significant differences in day of eye opening or of testicular descent. Although there were consistent differences in body weight, saline-treated animals were the lightest.

To assess the likelihood that the demasculinization was associated with reduced testosterone in adulthood, four separate groups of animals received perinatal treatments of HAL,  $\alpha$ -MT, APO, or saline identical to those that had produced behavioral demasculinization. Testosterone concentrations were assayed (18) in plasma obtained at 60 days of age. Although the  $\alpha$ -MT and APO groups had lower testosterone concentrations than did the HAL group, none of the drug groups was significantly different from the controls (Fig. 1d). Thus it appears that the behavioral demasculinization was not mediated by reductions in adult testosterone.

A single injection of dopamine antagonists can reduce masculine sexual behavior in adult rats (8, 9). However, we have now found deficits in sexual behavior of offspring resulting from long-term maternal treatment with drugs preferentially affecting DA activity. Contrary to our original hypothesis, all three HAL treatment regimens (HH, HS, and SH) impaired masculine behavior. Furthermore, increased (by APO), as well as decreased (by HAL and  $\alpha$ -MT), stimulation of postsynaptic neurons resulted in reduced masculine behavior. It would appear that any exogenous influences on DA activity during sensitive periods of development can compromise masculine development. It is possible that drug doses other than those administered here may be found to facilitate masculine development. The doses of HAL and  $\alpha$ -MT used here were chosen because they have been shown to produce enduring decreases (after prenatal administration) and increases (after neonatal administration) in DA receptor binding and in behavioral sensitivity to a DA agonist (12). Thus the consistent impairment by all drug treatments is intriguing. Demasculinization has also been reported after exogenously elevated testosterone as well as after testosterone deprivation (4).

The modes of action of perinatally administered drugs are multifaceted and are not well understood. Several drugs reported to demasculinize rodents have been found to reduce testosterone perinatally or during adulthood. However,

the lack of such suppression by HAL on fetal testosterone or by any of our perinatally administered drugs on adult testosterone appears to rule out simple changes in androgen levels as a factor in our results.

One possible mechanism may be altered neuronal growth or function. It has been suggested that the presence of monoamines one week before they are needed for synaptic transmission implies their use as trophic substances (21). Interference with monoamine activity perinatally has been reported to impair several measures of neuronal maturation (22). We suggest that the demasculinizing effects of perinatally administered drugs may be exerted directly on the neurons that regulate sexual behavior in adulthood and that these effects do not require a reduction of testosterone perinatally or in adulthood. Furthermore, since the alterations in receptor binding induced by prenatal HAL decreased steadily from 28 to 60 days of age (12), sexual impairment as late as 120 days of age may be related to permanently changed patterns of neural growth, synaptogenesis, or some factor other than receptor binding.

Drugs affecting the monoamines are widely prescribed for psychological disorders, hypertension, and emesis. Restricted drug intake is frequently advised during the first trimester of pregnancy, when major fetal organs are forming. However, neuronal growth and synaptogenesis occur later and continue after birth. Evidence of enduring behavioral deficits resulting from perinatal drug treatment in rats suggests caution in treating women with such drugs during pregnancy or lactation.

ELAINE M. HULL\*

J. KEN NISHITA†

DANIEL BITRAN

Department of Psychology,  
State University of New York,  
Buffalo 14226

SUSAN DALTERIO

Department of Pharmacology,  
University of Texas Health Science  
Center, San Antonio 78284

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13. Males were tested in their home cages with a receptive female for 30 minutes after the first intromission. Latency to each mount, intromission, and ejaculation was recorded. Intromissions per ejaculation, latency from first intromission to first ejaculation, and latency to first postejaculatory intromission were calculated.
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15. HH animals intromitted in 18 tests and ejaculated in 9 of them. HS animals intromitted in 17 tests and ejaculated in 9. SH males ejaculated in 15 of 21 tests with intromissions. SS animals ejaculated in 26 of 30 tests with intromissions.
16. Ovariectomized female offspring were injected with 4  $\mu$ g of estradiol benzoate 48 and 24 hours before a test and with 500  $\mu$ g of progesterone 4 hours before the test. Lordosis ratings on a 4-point scale were averaged over ten attempted mounts by the male on each of three weekly tests.
17. Open field ambulation was measured by the number of lines crossed in 10 minutes in a 1-m<sup>2</sup> box divided by lines into 16 equal squares. Catalepsy was tested 30, 60, 90, and 120 minutes after intraperitoneal injection of HAL (1 mg/kg). The animal's hind feet were placed on a line 13 cm behind and parallel to a plastic cage. The forepaws were placed on the 13-cm-high edge of the cage. The total number of seconds that the rat maintained this position over four tests was recorded.
18. Assays were performed with the technique of Dalterio and Bartke (5).
19. Animals given  $\alpha$ -MT intromitted in 39 tests and ejaculated in 26 of those. Animals given APO intromitted in 49 tests and ejaculated in 27. Animals given  $\alpha$ -MT plus APO intromitted in 46 tests and ejaculated in 31. Control rats ejaculated in 39 of 42 trials with intromissions.
20. Behavior was observed for 1 minute at 10-minute intervals for a total of 60 minutes after intraperitoneal injection of APO (1 mg/kg) and was rated as follows: 0, normal behavior; 1, occasional bursts of stereotyped sniffing or licking; 2, frequent bursts of sniffing, licking, or biting; 3, continuous sniffing, licking, or biting and restricted locomotion; and 4, continuous licking or biting and no locomotion. Catalepsy and stereotypy tests were separated by 2 to 4 days and were administered in counterbalanced order.
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\* To whom correspondence should be addressed.

† Present address: Department of Psychiatry, Duke University Medical Center, Durham, N.C. 27710.

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